Section 5: 510(k) Summary

1. Assigned 510(k) number

The assigned 510(k) number is K101454

JAN 28 2011

2. Company

Agendia BV
Science Park 406
1098XH Amsterdam
The Netherlands
Telephone : 31 20 462 1500

3. Contact

Guido Brink, Senior Director Regulatory Affairs and Quality Assurance

4. Date Prepared

May 21, 2010

5. Proprietary Name

MammaPrint®

6. Classification Name

Gene expression profiling test system, for breast cancer prognosis.

7. Common Name

Multivariate device for cancer prognosis

8. Classification

Class II, regulated under 21 CFR 866.6040, product code NYI

9. Predicate Device

Agendia BV's MammaPrint (k081092)

10. Device Description

The MammaPrint service is a microarray based gene expression analysis of a tumor. The analysis is based on several processes: isolation of RNA from frozen tumor tissue sections, DNA'se treatment of isolated RNA, linear amplification and labeling of DNA'se treated RNA, cRNA purification, hybridization of the cRNA to the MammaPrint microarray, scanning the MammaPrint microarray and data acquisition (feature extraction), calculation and determination of the risk of recurrence in breast cancer patients.

The MammaPrint analysis is designed to determine the gene activity of specific genes in a tissue sample compared to a reference standard. The result is an expression profile, or fingerprint, of the sample.

The correlation of the sample expression profile to a template (the mean expression profile of 44 tumors with a known good clinical outcome) is calculated and the molecular profile of the sample is determined (Low Risk, High Risk).

11. Intended Use

MammaPrint is a qualitative in vitro diagnostic test service, performed in a central laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess a patient's risk for distant metastasis (up to 10 years for patients less than 61 years old, up to 5 years for patients' ≥ 61 years).

The test is performed for breast cancer patients with Stage I or Stage II disease, with tumor size <= 5.0 cm and who are lymph node negative. The MammaPrint result is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors.

12. Performance Data (non-clinical)

Analytical performance

MammaPrint analytical (i.e., non-clinical) performance characteristics investigated comprise Precision and Reproducibility compared to the predicate device.

1 – Micro Array Scanners

In concordance with experiments performed for an additional scanner clearance in k080252, experiments were performed.

A selection of 25 slides performed as dual hybridizations from which MammaPrint Indices of the 1st scan was generated using the FDA cleared scanners (serial US45103019 and US22502555) were used, consisted of approximately 100 samples and 20 times the control samples LRC and HRC.

The samples and controls were analyzed during regular diagnostics. For the scanner validation these same 25 slides were scanned a second time using the new scanners (US810R3210 and US811R3213). Txt files generated as output were used to generate MammaPrint Indices following standard procedures. Subsequently MammaPrint Indices of both scans were compared. The hybridized samples included: three samples with either high, low, borderline results with repeated results were generated per sample. Additionally, control samples LRC and HRC were included. MammaPrint indices were compared between both scans using Passing and Bablok regression analysis and a comparison of the variance per scanner.

The difference between the mean, median and standard deviation for all samples levels between both scanners fall within the accepted variance of the predicate device of 1.96*0.030.

2 – Bio Analyzers

A selection of about 60 samples that cover the complete RIN measuring range was analyzed on the FDA cleared Bio-analyzers (Serial nr DE24802382 and DE54700497), as well as the new Bio-Analyzers (Serial nr DE72901757 and DE72902838).

Depending on the distribution of the data a statistical test was performed to determine if there is a significant difference in RIN measurements between both Bio-analyzers.

The RIN measurements of the samples on both Bio-Analyzers were collected. Subsequently the D'Agostino-Pearson test on the RIN differences of both analyzers showed that there a normal distribution ($p<0.0001$).

Therefore a Wilcoxon signed ranks test was used which showed that there was no significant difference in RIN measurements between the FDA cleared and New Bio-analyzers (p=0.46 and p=0.47 respectively).

3 – Central Laboratory sites Europe and U.S.

Validation of MammaPrint at the European and U.S. central laboratories was performed in two parts. All experiments were performed using FDA cleared equipment and in compliance with FDA cleared MammaPrint procedures.

PART 1: RNA ISOLATION

Samples were selected based on sufficient tissue material available for sectioning and isolation at the US laboratory (Lab 2). These samples have previously shown to generate acceptable quality of RNA at the Amsterdam laboratory (Lab 1). After isolation the concentration and RNA quality (RNA integrity number, RIN) was assessed using the Bioanalyzers; all values have to meet the standard quality controls for MammaPrint (RIN>7). Isolations were performed on three different days, twelve samples each day, in total 36 samples.

PART 2: AMPLIFICATION/LABELING AND HYBRIDIZATION

For validation of the labeling, amplification and hybridization steps of MammaPrint at the US lab (Lab 2), RNA from 99 samples was used. All samples have been previously subjected to a diagnostic MammaPrint test at the Amsterdam Lab (Lab 1). Based on the Amsterdam result the following result distribution was selected:

- High risk: n=54 (54.5%)
- Low risk: n=38 (38.3%)
- Borderline: n=7 (7.1%)

RNA was amplified, labeled and hybridized according to standard MammaPrint protocols on FDA cleared MammaPrint Low (HD) 8-pack array.

The 99 samples the standard control samples (Low Risk Control and High Risk Control) were analyzed. To show MammaPrint stability over time and to determine variation in MammaPrint Index, LRC and HRC were analyzed on each labeling day and on additional days resulting in 20 data points per control sample.

Statistical analysis that have been performed on the data are;

- Passing and Bablok regression analysis
- Bland & Altman analysis
- McNEMARS TEST
- Analysis on Control Pools: LRC AND HRC

The studies show that there is no significant difference in RNA quality of RIN measurement between Amsterdam (L1) and US lab (L2). All results comply with the predefined validation acceptance criteria as described in the validation plan.
Moreover when comparing of MammaPrint Index and Outcome, it is concluded that there is no significant difference in MammaPrint Indices between European / Dutch (L1) and US / California (L2) lab. All results comply with the predefined validation acceptance criteria as described in the validation plan.

14. Conclusion

MammaPrint is a clinically and analytically accurate prognostic marker for providing a risk assessment of distant metastasis of breast cancer when performed in either Agendia's European or US central laboratory.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Agendia BV
c/o Mr. Guido Brink
Director, Regulatory Affairs
Science Park 406
1098 XH Amsterdam
The Netherlands

JAN 28 2011

Re: k101454
Trade/Device Name: MammaPrint®
Regulation Number: 21 CFR§866.6040
Regulation Name: expression profiling test system for breast cancer prognosis
Regulatory Class: Class II
Product Code: NYI
Dated: December 23, 2010
Received: December 27, 2010

Dear Mr. Brink:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must

comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M. Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Section 4: Indications for Use Statement

Indications for Use Form

510(k) Number (if known): k101454

Device Name: MammaPrint®

Indications for Use:

MammaPrint is a qualitative in vitro diagnostic test service, performed in a central laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess a patients' risk for distant metastasis (up to 10 years for patients less than 61 years old, up to 5 years for patients' ≥ 61 years).

The test is performed for breast cancer patients with Stage I or Stage II disease, with a tumor size of ≤ 5.0 cm and lymph node negative. The MammaPrint result is indicated for use by physicians as a prognostic marker only, along with a other clinicopathological factors.

Prescription Use ___XX____ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Reena Philip

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510k k101454